PLASMA PROTEOMICS: OPTIMIZED EXPERIMENTAL DESIGN IN CARDIOVASCULAR DISEASE

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Atherothrombosis is the leading cause of death in the Western world. The pathophysiology of thrombosis is still unknown because its mechanisms are a network of molecules with multiple relationships among them. The use of new proteomic methodologies in the study of cardiovascular disease has generated new data of great interest and even the description of new biomarkers with diagnostic value. The challenge of working with biological samples such as plasma/serum, urine, saliva, CSF… is facing a dynamic range of several orders of magnitude (12 logarithmic scales in the case of plasma). Although there are robust analytical platforms and technologically advanced, it is a challenge to develop a workflow that allows both reduce the complexity of these samples and not lose any information. The advanced techniques of quantitative proteomics (SELDI-TOF, LC-MALDI-TOF/TOF, 2D-DIGE, etc) could address these samples but you need a "refinement" of them to gain access to known as "deep proteome ".

Several approaches are sequentially implemented to obtain an effective, fast and reproducible global workflow that could substantially improve the protein and peptide abundance/coverage in complex biological samples. For this purpose, a standard plasma sample is selected and depleted using TOP20 immunodepletion column (from Sigma-Aldrich) to remove the 20 highly abundant proteins.

A comparative analysis for several peptide fractionation techniques of the sample is performed (such as IEF fractionation (OFFGEL) and RP-HPLC at high pH) in order to increase peptide and protein identification. To evaluate both strategies we employ several mass spectrometry techniques: LC-MALDI-TOF/TOF and ESI-IT MS.

At the end, the tandem RP-HPLC at high pH with LC-MALDI-TOF/TOF is the defined workflow that will finally be applied to a real clinical assay to search plasma biomarkers associated with cardiovascular disease.